


ORIGINAL ARTICLE

Efficacy of enteric-coated tilmicosin granules in pigs artificially infected with *Actinobacillus pleuropneumoniae* serotype 2

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Abstract

Background: Porcine infectious pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (App) is one of the most serious infectious diseases in pigs and has brought huge economic losses to the world pig industry. The aim of this trial was to evaluate the effect of enteric-coated tilmicosin granule in the treatment and control of artificial infection of App.

Methods: Sixty Duroc and Yorkshire crossbred pigs (50 of which were artificially infected) were divided into six groups: BCG (Blank control group), ICG (Infection-only control group), HDG (High-dose enteric-coated tilmicosin granules), MDG (Medium-dose enteric-coated tilmicosin granules), LDG (Low-dose enteric-coated tilmicosin granules) and TPG (Tilmicosin premix drug control group). The cure rate, mortality, clinical respiratory score, body temperature score, weight gain, lung score and so on were recorded.

Results: The cure rate of HDG and MDG was as high as 90%, the mortality was 10%, and the clinical signs recovered quickly.

Conclusion: The results showed that enteric-coated tilmicosin granules had obvious therapeutic effect on artificial infection, which could reduce the damage caused by the disease and reduce the mortality.

KEYWORDS

Actinobacillus pleuropneumoniae, artificial infection, enteric-coated granule, pig, pleuropneumonia, Tilmicosin

Zhen Dong and Xu-Zheng Zhou are contributed equally to the work.

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1 | INTRODUCTION

Porcine infectious pleuropneumonia is a serious respiratory contagion caused by *Actinobacillus pleuropneumoniae* (App) (Vanden Bergh, Fett, Zecchinon, & Desmecht, 2008). The disease is transmitted through the respiratory tract and through secretions and exudates from coughing and sneezing, but contact transmission may be the main route of transmission (Chiers, Donn , Van Overbeke, Ducatelle, & Haesebrouck, 2002). App infections can be mixed infections involving *Pseudorabies virus* (PRV), *Pasteurella multocida*, *mycoplasma pneumoniae* (Wallgren, Artursson, Fossum, & Alm, 1993) and Porcine reproductive respiratory syndrome virus (PRRSV) (Pol, Van Leengoed, Stockhofe, Kok, & Wensvoort, 1997), which should be paid great attention to. There are many App virulence factors that play a role in swine infections. By analysing virulence factors and their immunogenic components (Dubansky, 2000), it was found that haemolytic toxin, capsular polysaccharide, and lipopolysaccharide play very important roles in the pathogenesis of pleuropneumonia. App is also a major immunogen (Haesebrouck, Chiers, Van Overbeke, & Ducatelle, 1997). Because of the rapid onset and high fatality rate of the infection, porcine infectious pleuropneumonia has caused huge economic losses to the pig industry (Zimmerman, Karriker, Ramirez, Schwartz, & Stevenson, 2012).

Currently, pigs with infectious pleuropneumonia are mainly treated with antibiotics. Tilmicosin, 20-deoxy-20-(3,5-dimethylpiperidin-1-yl) desmycosin, a synthetic derivative of tylosin, is a macrolide antibiotic for livestock and poultry (Debono et al., 1989). It has a similar molecular structure and antibacterial spectrum to tylosin (Kirst et al., 1989). According to research, tilmicosin has a good inhibitory effect on Gram-positive bacteria and some Gram-negative bacteria, mycoplasma, spirochetes, etc. (Inamoto et al., 1994). Additionally, it has a stronger antibacterial activity against App, *Pasteurella pneumoniae*, and mycoplasma than tylosin (Hong-Sheng et al., 2006). Tilmicosin reached its maximum blood concentration quickly in pigs and was eliminated very slowly (T_{\max} (h) = 3.53 ± 0.66 , $t_{1/2\beta}$ (h) = 43.53 ± 8.17) (Zhang, Zhao, Liu, Liu, & Li, 2017). Due to its unique antibacterial activity and pharmacokinetic characteristics, it has been widely used in the prevention and treatment of infectious diseases, especially respiratory diseases and mastitis in lactating animals (Shen et al., 2005).

At present, the tilmicosin premix products that are being marketed are mainly used in swine feed mixture. Because the bitter taste of this type of preparation is not palatable to pigs, it is generally necessary to add sweeteners or other additives to increase the palatability when the mixture is fed to pigs. To improve the palatability and efficacy of tilmicosin, we developed enteric-coated tilmicosin granules. To provide evidence for clinical use of the enteric-coated tilmicosin granules for treating infectious pleuropneumonia, the clinical efficacy in the treatment of swine artificially infected with App was evaluated in our laboratory.

2 | MATERIAL AND METHODS

2.1 | Experimental drug

Experimental drug: enteric-coated tilmicosin granules, grey-white symmetrical microencapsulated granules (the average particle size is about 1 mm), insoluble in water, coated with acrylic resin, containing 20% of the active ingredient tilmicosin, produced by Ringpu Biopharmaceutical Co., Ltd. (Tianjin, China), batch number: 121,001.

Control drug: tilmicosin premix, white crystal, bitter taste, soluble in water, containing 20% of the active ingredient tilmicosin, 1,000 g/bag, produced by Ringpu Biopharmaceuticals Co., Ltd. (Tianjin, China), batch number: 20,140,404.

2.2 | Randomized clinical trial

The study was performed as a randomized clinical trial. Data were collected from April to October 2014. The study was approved by the Tianjin Rural Work Committee and the Ethics Committee of Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS (TJRWC-Veterinary drug test license no.2014-1/ SYXK 2014-0002). And the trial is in accordance with the CONSORT (Consolidated Standards of Reporting Trials) regulations (Campbell, Elbourne, & Altman, 2012; O'Connor, Sargeant, Gardner, Dickson, & Torrence, 2010) and Chinese Laboratory animal—Guideline for ethical review of animal welfare (GB/T 35,892).

Double-blinding method was used in the selection of pigs, which was suitable for clinical trials. All these actions were performed by third-party personnel. The numbering of pigs in the group was randomly generated. Considering the possibility that drugs may have a greater impact due to gender differences, sex as a stratification factor makes them as similar (balanced) as possible in each group distribution. Simple randomization is used for each layer (male or female). Personnel who performed both the artificial infection test and the clinical symptom observation were trained, and multiple observations were made during the trial to ensure the authenticity of the results. Each section of the test was carried out by a separate person to ensure quantity accuracy. Randomisation was done before the test.

2.3 | Experimental animals

The study was carried out in the Da Wa mountain test base of Lanzhou Institute of Husbandry and Pharmaceutical Sciences. A total of 60 pigs were selected from a specialized pig breeding farm in Gansu Province. The pigs were crossbred Duroc (male parent) and Yorkshire (female parent), aged 6–7 weeks, weighing about 9–11 kg, half of which were male and half of which were female (Clayton & Collins, 2014). And swine herd was in different health state according to Chinese Laboratory animals-General requirements for animal experiment (GB/T 35,823). All pigs were foundation level, and they had not been vaccinated with App vaccine. According to the

standard livestock nutrition requirements, a basic diet without any antibiotics was prepared. The basic diet formula consists of two parts, viz, the principal ingredient (65% maize, 10% wheat bran, 20% soybean meal, and 5% fish meal) and the additive (0.9% calcium carbonate, 1.4% calcium bicarbonate, 0.1% salt, 0.1% multi-vitamins, and 2% colza oil residue).

In the experimental groups, different amounts of experimental drugs were added to the basic diet. Swine herds were fed in different but contiguous hog pens, which had four-fifths solid floor and one-fifth slatted floor. Before the experiment, the detection of *A. pleuropneumoniae* in the pigs and a 7-day period of health observation were carried out.

Before the experiment began, each pig was weighed and they were then divided into six groups (according to similar body weight and gender) with 10 pigs in each group, viz, BCG, ICG, LDG, MDG, HDG, and TPG.

2.4 | Bacteria

Local App serotype 2 strains of China and diagnostic antigens of *A. pleuropneumoniae* were provided by the Lanzhou Veterinary Institute of CAAS.

2.5 | Experimental instruments and reagent

The following instruments were used in the experiments: OLYMPUS AU2700 automatic biochemical analyser (Olympus Optical Co., Ltd., Tokyo, Japan), biological microscope (Leica Microsystems Inc., Buffalo Grove, Illinois, USA), ultraviolet spectrophotometer (model 722; Shanghai Precision Scientific Instruments Co., Ltd., Shanghai, China), low-speed centrifuge (Beijing Medical Centrifuge Factory, Beijing China), automatic blood cell analyser (Shenzhen Mindray Biomedical Electronics Co., Ltd., Guangdong, China), automatic dyeing machine (Leica Microsystems Inc., Buffalo Grove, Illinois, USA), rotary automatic slicer (Leica Microsystems Inc., Buffalo Grove, Illinois, USA), centrifugal tubes (Thermo Fisher Scientific Co., Ltd, Boston, Massachusetts USA), and slides and cover slides (Citotest Labware Manufacturing Co., Ltd, Jiangsu, China). Haematological and biochemical reagents (Changchun Huili Biotech Co., Ltd., Jilin, China) were obtained, and ultrapure water was produced for the experiments.

2.6 | Methods

2.6.1 | Bacterial culture

According to the corresponding culture mode of bacteria, freeze-dried *A. pleuropneumoniae* strains were revive and inoculated in TSB liquid medium (Rogers, Eaves, Blackall, & Truman, 1990). The bacterial solution was diluted with TSB liquid medium, a plate count was carried out, and the count results were used to determine the volume of bacterial suspension used to infect the pigs.

2.6.2 | Methods of administration and artificial infection

Through the pre-experiment, it was determined that the minimum lethal dose was between 3.52×10^7 CFU \sim 1.29×10^8 CFU/ml and the infection dose was 4ml. The inoculum size of this experiment was 3.24×10^7 CFU/ml \times 4 ml. BCG was inoculated with the same volume of normal saline (NS). Obvious clinical symptoms such as elevated body temperature (TEMP) appeared about 4 hr after artificial infection. The pharmaceuticals were then given different doses for 15 days according to the designed trial scheme. The drug was stopped after 15 days and the basic daily feed without drugs was used and continued to be observed until the 20th day. Artificial infection and administration methods are detailed in Figure 1.

2.6.3 | Body temperature measurement and clinical examination

Rectal temperature of each pig (all survival pigs) was measured at 13 time points, 2–144 hr before and after infection. At the same time, all survival pigs were clinically examined. Some clinical symptoms, including cough, fever, mental status, vomiting, and etc. were observed and recorded.

2.6.4 | Haematology and blood biochemistry

Haematology and blood biochemical examination were both conducted at 24 hr after infection and the 5th day after drug withdrawal (20th day after infection). The results were used as one of the objective measures to judge the success of artificial infection and the efficacy of treatment.

At 24 hr after artificial infection, five pigs were randomly selected from each group to obtain blood samples. In addition, on the 20th day after infection (at the end of the experiment), five pigs were selected according to the availability of surviving pigs in each group (in the infection-only control group, there were only two surviving pigs, so the sample size regarding blood samples was too small to be statistically analysed). The collected blood samples were anticoagulated with heparin, and routine blood tests were carried out using an automatic blood analyser. The haematological parameters were leukocyte count (WBC), lymphocyte count (LYH), neutrophil count (NEU), platelet count (PLT), red blood cell count (RBC), haemoglobin content (HGB), mean platelet volume (MPV, in fL) and platelet distribution width (MPD, in fL).

For blood biochemical examination, whole blood samples were collected under the same conditions as described previously, and the serum samples were obtained after blood coagulation. The biochemical parameters were albumin (ALB), total protein (AP), globulin (GLB), direct bilirubin (DBIL), total bilirubin (TBIL), indirect bilirubin (IBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen (BUN) and creatinine (GREA).

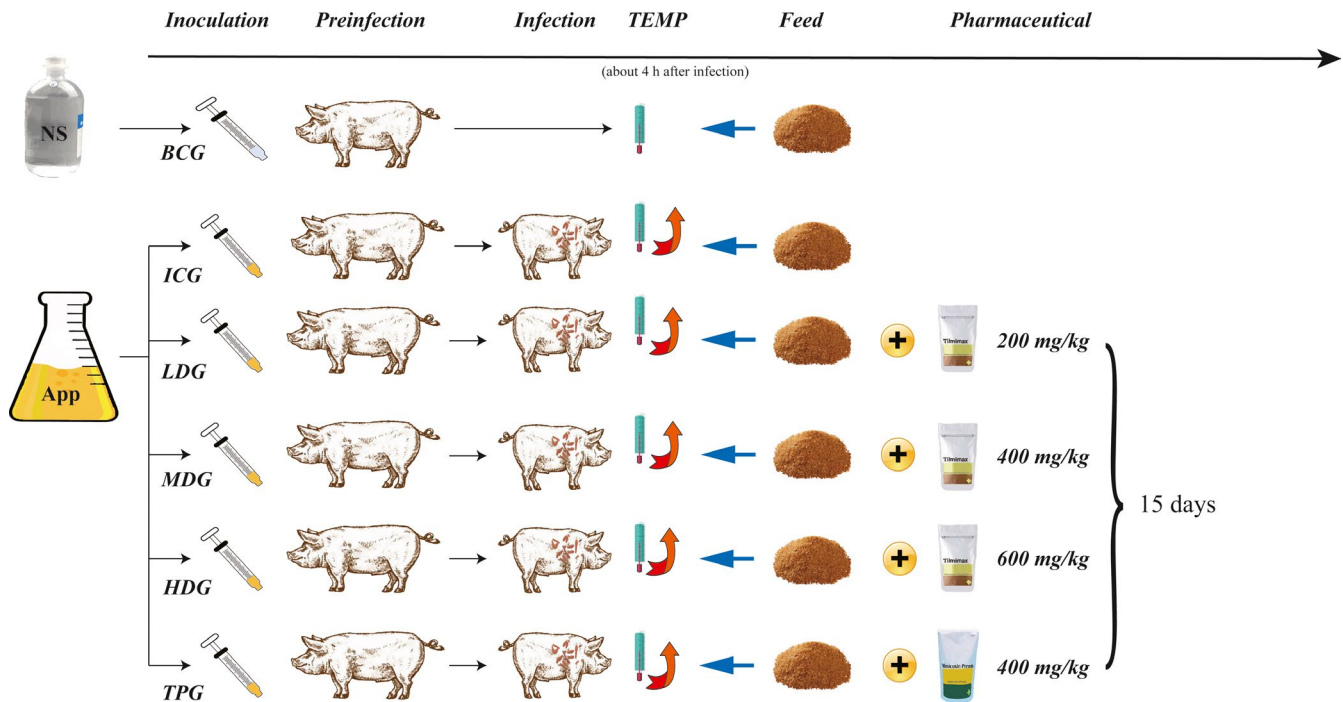


FIGURE 1 Artificial infection and administration methods

2.6.5 | Histopathological examination

General pathology examination was performed on the pigs that died during the experiment. According to Farm Animal Welfare requirements for Pigs (China association for standardization, 2014), all the surviving pigs were killed by bloodletting at the end of the experiment after being stunned by electric shock. The examination involved observing whether the main organs appeared to have undergone macroscopic swelling, shrinkage, colour and hardness changes, purulence, bleeding and other lesions. Liver, lung and spleen tissues were collected and applied to plate culture medium to detect the presence of App. The lung, liver, spleen and other tissues with suspected pathological changes were stored in neutral formalin buffer solution, and further histopathological examination was carried out after treatment with routine pathological process, such as washing, trim, dehydration, staining and optical observation.

2.6.6 | Statistical analysis

The experimental data were statistically analysed by SPSS 19.0 for Windows (SPSS Inc.). The statistical significance of the differences in mortality rate, cure rate, effective rate, relative weight gain rate, haematology and blood biochemistry were tested. Changes in the number of animals that die are considered. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); Means of two continuous normally distributed variables were compared by independent samples *T* test. All significant results ($p < .05$) were labelled and further analysed (Habibzadeh, 2017).

3 | RESULTS

3.1 | Clinical observation results

All the pigs were weighed before the experiment and 20 days after infection. The results show that the growth of pigs in the four experimental groups and the infection-only control group were affected to varying degrees due to artificial infection. In particular, the surviving pigs in the infection-only control group grew slowly, were emaciated, and had a significantly lower weight gain ($p < .01$) than those in the healthy control groups and the four experimental groups. However, there was no significant difference in relative weight gain rates among the four experimental groups or between each of the four experimental groups and the healthy control group ($p > .05$). Detailed weight and weight gain data of each group are shown in Table 1.

The experimental groups with the addition of drugs had a good cure rate. Among them, the cure rate of MDG and HDG was as high as 90%. The therapeutic effects of each group are shown in Table 2.

From the curve of body temperature change in Figure 2, it can be seen that the body temperature of piglets in the ICG and the four treatment groups began to increase at about 4 hr ($0.1 \leq 0.4^\circ\text{C}$), reached the peak at about 6 hr ($1.0 \leq 1.7^\circ\text{C}$), and then began to slow down. Among them, the body temperature of piglets in the four treatment groups decreased significantly, and was significantly lower than that in the ICG ($p < .05$). Although the body temperature increased at about 10 hr, there were no obvious symptoms of fever. Although the body temperature of piglets in the ICG decreased briefly after 6 hr, it remained at a high level until 12 hr before the body temperature began to decrease gradually.

TABLE 1 20-day weight gain statistics of feed-fed with tilmicosin

Group	IMW/kg	TMW/kg	MWG/kg	RWGR/%
BCG	9.90 ± 2.26	14.06 ± 2.28	4.16 ± 0.70	100.00
ICG	9.80 ± 2.19	12.05 ± 0.97	2.68 ± 0.54*	64.40
HDG	9.82 ± 2.09	13.73 ± 1.79	3.76 ± 0.67	90.38
MDG	9.85 ± 1.94	14.13 ± 1.68	4.08 ± 0.48	98.10
LDG	10.1 ± 2.33	14.2 ± 2.28	3.97 ± 0.51	95.43
TPG	10.7 ± 1.97	15.63 ± 1.99	3.91 ± 0.60	94.00

Note: RWGR, $100 \times (W_{\text{terminal}} - W_{\text{initial}}) / W_{\text{initial}}$.

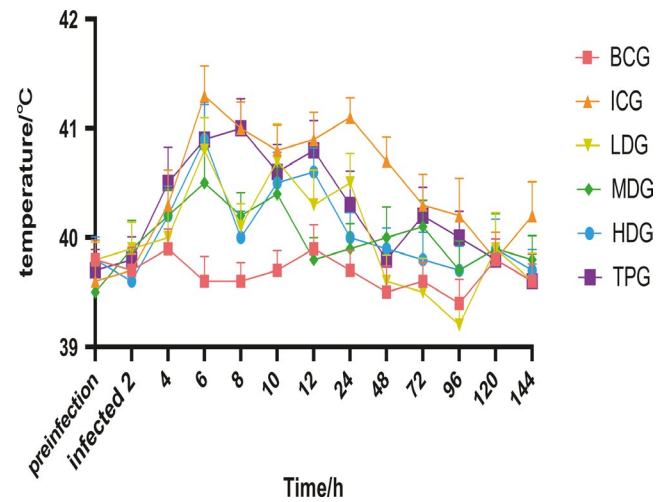
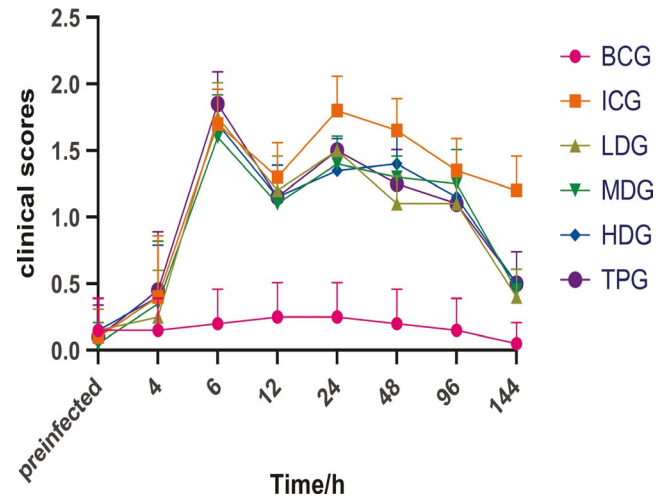
Abbreviations: IWM, Initial mean weight; MWG, Mean weight gain; TW, Terminal mean weight; RWGR, relative weight gain rate.

* $p < .05$.

The clinical score criteria refer to previous reports (Sibila, Aragón, Fraile, & Segalés, 2014). Clinical score = (Respiratory score + Rectal temperature)/2. The clinical scores of the four treatment groups gradually decreased to close to the level of the BCG after the acute phase of infection. The clinical score of the ICG has been maintained at a high level ($p < .05$). The specific changes are shown in Figure 3.

3.2 | Haematology and blood biochemistry results

At 24 hr after infection, the WBC, NEU, PLT and HGB of each group were higher than that of BCG ($p < .05$) (Figure 4a). On the 20th day, the WBC, NEU and HGB of each group had decreased to close to BCG. There was also a significant decrease in PLT compared with 24 hr after infection with almost no difference between HDG and BCG ($p > .05$) (Figure 4b). After 24 hr of infection, the TBIL, ALT, AST, BUN and CREA of each experimental group were significantly different from those of BCG ($p < .05$) (Figure 4c). On the 20th day, there was no significant difference between all the indexes and BCG (Figure 4d). Note: * VS BCG ($p < .05$).

**FIGURE 2** Changes in body temperature after infection in pigs in each experimental group**FIGURE 3** Clinical score statistics of pigs in each experimental group**TABLE 2** Summary of curative effect of each treatment group

Group	N	Morbidity (%)	Disease time (h)	Death rate (%)	Death time (h)	Effective rate (%)	Cure rate (%)	Survival rate (%)	N.App
HDG	10	100	4-5	10	144	90	90	90	0
MDG	10	100	4-5	10	120	90	90	90	0
LDG	10	100	4-5	20	48-96	80	70	80	1
TPG	10	100	4-6	20	48-102	80	80	80	0
ICG	10	100	4-5	60	22-90	40	0	40	4
BCG	10	0	-	0	-	-	-	100	0

Note: Cure rate = Number of pigs without detection of App/The total number of pigs in the group.

Effective rate = Number of pigs with reduced clinical symptoms and detectable App/The total number of pigs in the group.

Dead rate = Number of dead pigs/The total number of pigs in the group.

Survival rate = Number of undead pigs/The total number of pigs in the group.

N.App = Number of live pigs that can detect App.

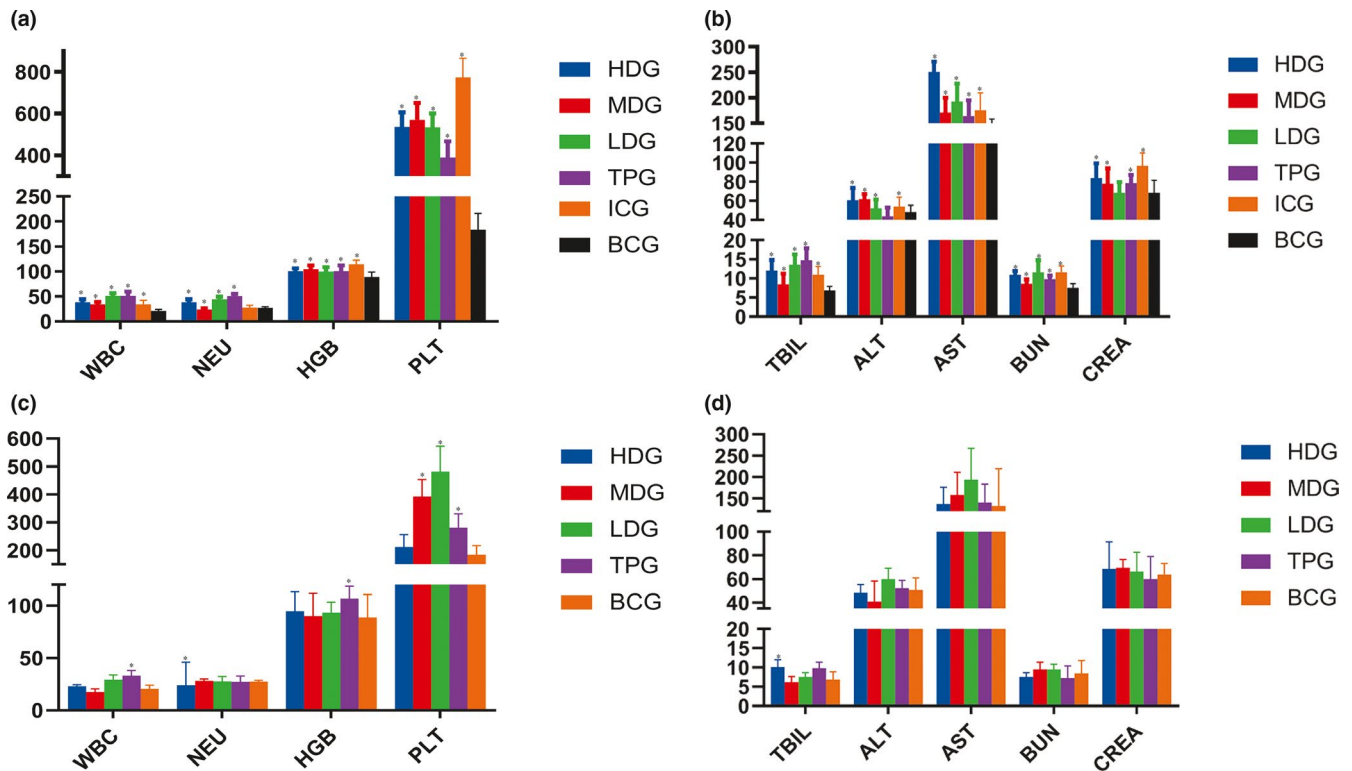


FIGURE 4 (a) Haematological indexes of experimental pigs at 24 hr after artificial infection. (b) Serum biochemical indexes of experimental pigs at 24 hr after artificial infection. (c) Haematological indexes of experimental pigs on 20th day after artificial infection. (d) Serum biochemical indexed of experimental pigs on the 20th day after artificial infection

3.3 | Histopathological results

3.3.1 | General observation of slaughtering

Slaughterhouse Pleurisy Evaluation System (SPES) is a subjective, fast and simple lung scoring system that provides information on the extension, and localization of App-like lesions (Fraile, Alegre, López-Jiménez, Nofrarias, & Segalés, 2010). According to this scoring system, pigs in ICG can get 2 points. One pig in LDG and one in TPG scored 1 point each, and all the other pigs can be thought to have scored 0.

3.3.2 | Histopathological examination

In ICG, the liver showed extensive and severe congestion, high oedema, extensive cell degeneration and systemic blood circulation disorder. The pigs in the treatment groups showed a small amount of congestion and mild cell deformation, but there is a higher oedema shown in the liver for HPG. The lungs of the infected pigs became lobar pneumonia, accompanied by suppurative pneumonia and interstitial pneumonia. The specific manifestations of the lesions are High pulmonary oedema, congestion, necrosis and shedding of tracheal and bronchial epithelial cells, cellulose necrosis of tube wall, and acute inflammatory cells, including monocyte, macrophage suppurative pneumonia. The pigs in each treatment group showed different degrees of interstitial pneumonia. The spleen of pigs in ICG showed congestion, lymphocyte necrosis in the white pulp area, and

the boundary between the red pulp and the white pulp was not clear. In the treatment groups, except for a small amount of necrosis at the edge of the white pulp in HDG, the other groups were normal. The pathological section pictures are shown in Figure 5.

4 | DISCUSSION

Porcine infectious pleuropneumonia has caused great trouble to the pig industry all over the world. Because of its rapid onset, a large number of pigs can usually be seen to die after the outbreak, and pigs passing through the acute phase will usually show chronic infection and become pathogen carriers. There are many serotypes of App, and the virulence is also very different. the virulence of serotype 1 is the highest, followed by serotype 2 and type 7 (Rogers et al., 1990). Pigs die quickly after App serotype 1 infection and usually do not have time to treat them. App serotype 2 has been detected more and more recently and has also become the main pathogen (Ohba et al., 2010; Sarkozi, Makrai, & Fodor, 2018). In the event of an acute outbreak of App, it can be treated with tilmicosin injection (Papatsiros et al., 2018), but if it occurs on a large scale, the injection will greatly increase the cost and require a large amount of labour. Pigs are often chronically infected and App is detected at slaughter (Ohba et al., 2009). Therefore, it is the most economical and feasible way to mix drugs and feed, or to put them in water. Enteric-coated tilmicosin granules can cover up the bitterness of the drug itself and solve the

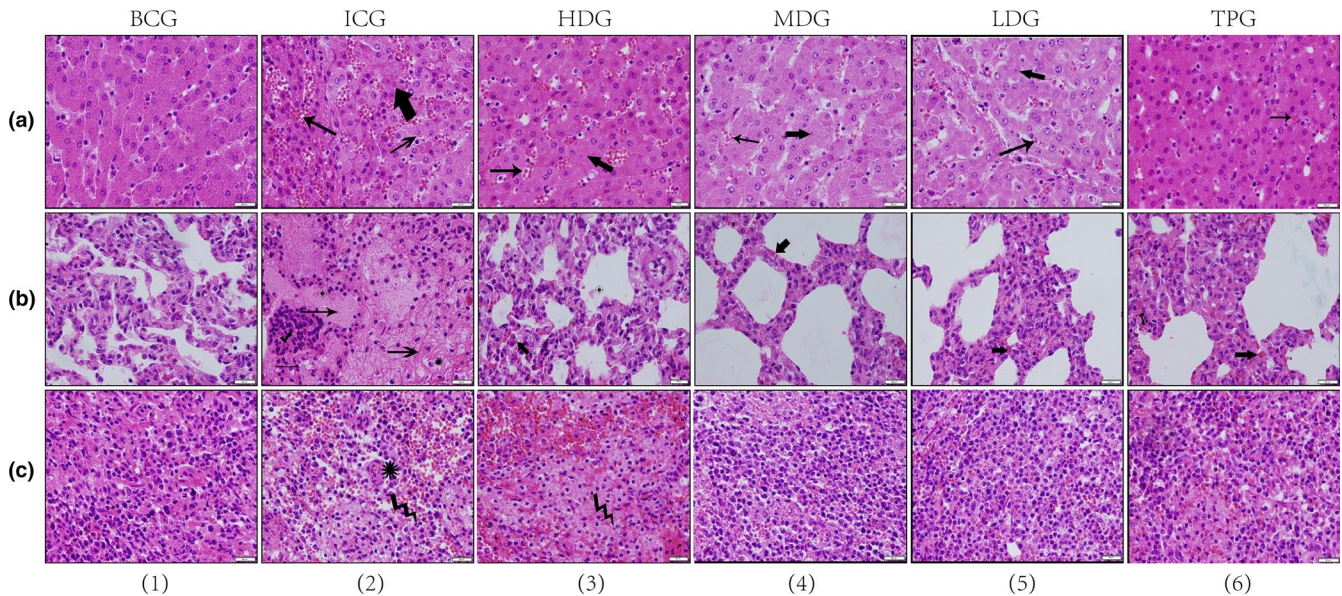


FIGURE 5 a (liver): long arrow for congestion, short arrow for cell necrosis, and thick arrow for cell oedema; b (lung): long arrow for pulmonary oedema, short arrow for cellulose necrosis, and thick arrow for interstitial pneumonia; c (spleen): asterisks for congestion, and lightning for lymphocyte necrosis. Samples were collected from autopsies on the 20th day after infection and death in the course of infection. HE stain (40×10)

problems caused by anorexia and poor palatability in sick pigs to a great extent. Previous studies in our laboratory have shown that enteric-coated tilmicosin granules provide higher relative bioavailability in pigs ($AUC_{(0-t)}$ of enteric-coated tilmicosin granules is $(19,371.24 \pm 1,057.39) \text{ ng}\cdot\text{mL}^{-1}\cdot\text{h}$; $AUC_{(0-t)}$ of tilmicosin premix is $(11,272.99 \pm 1,222.31) \text{ ng}\cdot\text{mL}^{-1}\cdot\text{h}$, relative bioavailability = $171.83 \pm 8.16\%$) (S. Gong, B. Li, X. Zhou & J. Zhang, unpublished) (Li et al., 2017; Xiong et al., 2019).

This experiment proved that enteric-coated tilmicosin granule has a good therapeutic effect on App infection. The cure rate of MDG and HDG was as high as 90%. Compared with ICG, the survival rate was significantly improved ($p < .05$). From Table 2, the treatment results of TPG and LDG are similar. The results of MDG and HDG were slightly better than those of TPG, but there was no significant difference between them ($p > .05$). After drug intervention, the clinical signs, growth performance, bacterial examination, pathological damage and other parameters of pigs have been well improved. After eating, the clinical score of pigs in the treatment group decreased gradually with time, and there was no significant difference between the treatment group and BCG ($p > .05$). During the autopsy, only one pig with a lung score of 1 was examined by LDG and TPG in the treatment group. Through histopathological examination of the lungs, ICG's lungs were severely damaged, with the highest score of 5 according to previous reports, compared with a score of between 1 and 3 in the treatment groups (Ashcroft, Simpson, & Timbrell, 1988; Mikawa, Nishina, Takao, & Obara, 2003). These therapeutic effects have been confirmed in previous studies (Hoflack, Maes, Mateusen, Verdonck, & De Kruijff, 2001; Mateusen, Maes, Hoflack, Verdonck, & De Kruijff, 2001; Paradis et al., 2004).

Clinicopathological results showed that App infection can cause severe inflammatory reaction (Bossé et al., 2002; Kiorpes, Mirsky, MacWilliams, Bäckström, & Collins, 1989). Tilmicosin can

significantly reduce these adverse reactions. There is some evidence to help explain the clinical efficacy of tilmicosin. Tilmicosin can migrate macrophages and neutrophils to the infected site (Scoreaux & Shryock, 1998) and stimulate lysosomal enzyme secretion (Diarra, Malouin, & Jacques, 1999). In addition, tilmicosin has been shown to have a peripheral analgesic effect that can help relieve the pain caused by the disease (El-Mahmoudy & Gheith, 2016). The results of clinical chemistry and pathology show that the infection has serious damage (cell necrosis) to the liver. The change of liver function is also an important diagnostic index after pneumonia infection (Huang et al., 2018). Liver function has returned to normal after treatment. Previous reports have also demonstrated that the activation and regulation of multiple genes in the liver play an important role in innate immunity after App infection (Skovgaard, Mortensen, Boye, Hedegaard, & Heegaard, 2010). The recovery of liver function also helps to eliminate infection. And tilmicosin did not have a negative effect on animals (Altunok et al., 2010), except for the HDG. This may be due to the side effects, which is a typical response to high doses of an antibiotic compound (Roberts, 2006).

The results of this experiment can show that enteric-coated tilmicosin granules can effectively treat App infection and effectively remove App in vivo. In addition, it has been previously reported that high concentrations of tilmicosin can cause immunosuppression in the body (Guan et al., 2011), so do not use it with vaccines in the treatment or prevention of diseases to avoid reducing its titre.

5 | CONCLUSION

The results showed that administration of enteric-coated tilmicosin granules had obvious effects on artificial infection-induced

porcine *Actinobacillus* involving pleuropneumonia. The treatment could kill and clear the pathogenic bacteria *in vivo*, delay the course of the disease, resist tissue damage, and reduce the overall damage caused by disease. It also reduces the mortality rate. Enteric-coated tilmicosin granules are safe to use at the recommended dosage. Considering the therapeutic effects and side effects, we support that 400 mg/kg feed mixture for 15 days is effective.

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CONFLICT OF INTEREST

Author Xiao-bin Meng was employed by the company Ringpu Biopharmaceutical Co., Ltd., Tianjin, P. R. China. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

XZ and ZD conceived and design the experiments. JY developed the method. XZ, JC and WW performed the experiments and made records. XZ agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. HS and XJ carried out the bacterial tests. ZD carried out the histopathological examinations. FS and BL analysed the data. ZD and WW interpreted the literature references and provided the background information. XB was responsible for drug palatability test. ZD wrote the paper. JY applied for the funds and provide approval for publication of the content.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The Chinese National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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